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Effects in the Mouse and Rat of Prenatal Exposure to Arsenic

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Initial experiments involving mouse development employed single IP injections of 45 mg/kg sodium arsenate on one of days 6-12 of gestation and produced a spectrum of developmental defects. Embryotoxicity was indicated by high prenatal mortality and decreased fetal weights. A chelating agent, 2,3-dimercaptopropanol (BAL), was then employed in an attempt to alleviate the adverse effects of prenatal arsenate. BAL was administered 4 hr before, concurrently with, or 4 hr after arsenate. All BAL treatments diminished arsenate-induced gross malformations and growth retardation; the concurrent treatment alleviated skeletal malformation. Injection of rats IP with arsenate has also been reported to result in teratogenicity, including renal agenesis. Further reports indicated that 40 mg/kg arsenate administered to mice by gavage on days 9–11 increased prenatal mortality, reduced fetal weights, and was associated with minor malformations. According to our recent work, however, single oral doses of arsenate must be around 120 mg/kg to cause prenatal toxicity. Multiple doses of 60 mg/kg on 3 days had little effect. Sodium arsenite has also been found to be fetotoxic and teratogenic. Such effects were seen at IP doses of 10–12 mg/kg.

Introduction

Although both man and domestic animals may be exposed to a variety of arsenic compounds, only a few such compounds have been investigated with regard to possible prenatal effects. The initial work describing embryotoxic and teratogenic effects was done with chicken embryos by Ancel (*1*), who used disodium methylarsenate, and by Ridgeway and Karnofsky (*2*), who tested various arsenic salts. Most subsequent reports have dealt with effects of arsenic in the hamster, as discussed by Ferm at this conference (*3*), and in the mouse and rat, which are the subject of the balance of this report.

Materials and Methods

For all experiments done in our laboratory, random-bred albino Swiss-Webster mice of the CD-1 strain were obtained from Charles River Mouse Farms and maintained on an *ad libitum* diet of Wayne Lab Blox. The day on which a vaginal plug was found was considered day 1 of gestation. Arsenic treatments consisted of intraperitoneal injection or gastric intubation on the gestation day indicated. Distilled water was used as the solvent for dibasic sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) or for sodium arsenite ($NaAsO_2$). One experiment also involved BAL (British antilewisite or 2, 3-dimercaptopropanol). This chelating agent was disolved in corn oil and injected subcutaneously in the nape of the neck. A 50 mg/kg dose of BAL was administered on gestation day 9, either 4 hr before (B/A), currently with (A + B), or 4 hr after (A/B) 40 mg/kg arsenate. All mated females were sacrificed on gestation day 18. Observations were then made of prenatal mortality, fetal malformations and fetal weights. One third of each litter was cleared and stained for skeletal observations (*4*).

Results and Discussion

Our initial work with arsenic (*5*) involved IP injection of mice with 25 or 45 mg/kg sodium arsenate on one of gestation days 6–12. Treatment at the lower dose level had no discernible effect, but the high dose caused increased prenatal mortality, decreased fetal weights, and a spectrum of gross and skeletal abnormalities (Table 1). Some of the major defects observed and their relative frequencies are listed in Table 2. Such results are indicative of a

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Table 1. Effects of sodium arsenate on fetal development in mice: single IP injections on one of days 6-12 of gestation.[a]

Day of treatment[b]	No. pregnant	Dead or resorbed, %	Fetal weight, g ± SE	Grossly malformed, %
6	10	51	0.88 ± 0.02	2
7	8	37	0.67 ± 0.03	34
8	11	56	0.87 ± 0.02	36
9	8	60	0.61 ± 0.03	63
10	10	51	0.79 ± 0.03	26
11	10	69	0.94 ± 0.03	8
12	8	78	1.05 ± 0.02	0
Control[d]	37	4	1.05 ± 0.01	1

[a]Adapted from Hood and Bishop (*5*).
[b]Pregnant females received 45 mg/kg sodium arsenate.
[c]Killed on day 18 of gestation. Treatment on days 6-11 resulted in decreased fetal weights when compared with controls ($p < 0.05$).
[d]Control mice were injected with distilled H_2O on one of gestation days 6–12.

general rather than a specifically acting teratogen, as the anomalies seen involve a wide variety of developmental defects.

Frequent exencephalies and eye and rib malformations similar to those in the mouse have also been seen in the hamster (*6*), but in the rat, according to Beaudoin (*7*), arsenate produced a preponderance of skeletal defects along with renal agenesis and anophthalmia, and only a relative few exencephalies.

An additional study (*8*) was done with arsenate to determine if the chelating agent, BAL, could protect against the prenatal effects of arsenate. As can be seen in Table 3, results of arsenate treatment alone were similar to those previously discussed (*5*). BAL alone had no significant adverse effects, although at the much higher dose of 1200 mg/kg, BAL has been reported (*9*) to be teratogenic in mice. In all cases, however, BAL treatment diminished the incidence of arsenate-induced gross malformations and growth retardation. The concurrent treatment (A + B) also alleviated the skeletal malformations associated with arsenate treatment, while the other two treatments decreased the severity (though not the incidence) of such defects. It is possible that the BAL was acting to increase the rate of arsenic excretion and thus reduce embryonic exposure. The two sulfhydryl groups of the BAL molecule form a stable ring with arsenite ions, while the hydroxyl group makes the complex water soluble and excretable in the urine. Thus, if there is an

Table 2. Sodium arsenate-induced fetal anomalies in mice: day of treatment versus response.[a]

	Affected animals with treatment of various gestation periods						
	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Anomaly	N[b] %	N %	N %	N %	N %	N %	N %
Exencephaly	0/56 0	2/62 3	20/64 31	25/46 54	0/56 0	0/37 0	0/19 0
Shortened jaws	0/56 0	3/62 5	8/64 12	21/46 46	1/56 2	0/37 0	0/19 0
Anophthalmia	0/56 0	1/62 2	7/64 11	4/46 9	0/56 0	0/37 0	0/19 0
Open eye	3/56 5	12/62 19	9/64 14	9/46 20	1/56 2	0/37 0	0/19 0
Umbilical hernia	0/56 0	11/62 18	0/64 0	4/46 9	0/56 0	0/37 0	0/19 0
Malformed limbs	0/56 0	0/62 0	0/64 0	1/46 2	4/56 7	1/37 3	0/19 0
Missing or short tail	0/56 0	0/62 0	0/64 0	3/46 7	0/56 16	1/37 3	0/19 0
Twisted tail	1/56 2	0/62 0	0/64 0	1/46 2	5/56 9	1/37 3	0/19 0
Malformed ribs	0/17 0	0/17 0	5/18 28	11/11 100	0/15 0	0/12 0	0/ 7 0
Fused vertebrae	0/17 0	0/17 0	0/18 0	11/11 9	11/15 73	0/12 0	0/ 7 0

[a]Adapted from Hood and Bishop (*5*).
[b]Values (*N*) represent the number of affected animals/total number of fetuses examined.

Table 3. Effects of BAL on arsenate-induced fetal death and malformation in mice.[a]

Treatment	No. of pregnant mice	Dead or resorbed fetuses,%	Fetal weight, g ± SE	Grossly malformed fetuses, %	Skeletal malformations, %
A	16	29[c]	0.78 ± 0.01	54	77.7[c]
B	15	9[d,e]	0.96 ± 0.02[c,d]	0[d]	0.0[d]
B/A	15	19[c,e]	0.92 ± 0.01[d]	17[c]	69.6[c]
A+B	18	14[c,e]	0.93 ± 0.01[d]	10[c]	0.0[d]
A/B	15	27[c]	0.95 ± 0.02[c,d]	9[c]	47.8[c]
+ Control	15	9[d,e]	0.95 ± 0.02[c,d]	0[d]	0.0[d]
− Control	12	5[d]	1.01 ± 0.01[c]	0[d]	0.0[d]

[a]Adapted from Hood and Pike (*8*).
[b]For treatments, see materials and methods section.
[c,d,e]Values in a category sharing the same superscript were not significantly different ($p < 0.05$) according to the Newman-Keuls test.

appreciable degree of *in vivo* interconversion between arsenate and arsenite, the arsenite could be continually removed as it is produced, having the effect of decreasing the arsenic levels present in the system.

A study involving the IP injection of sodium arsenate in pregnant rats has been reported by Beaudoin (*7*). Treatment of Wistar rats on one of gestation days 8–13 with a dose of 50 mg/kg invariably resulted in embryonic mortality. A dose of 20, 30, or 40 mg/kg caused increased mortality as well as developmental defects. Burk (*10*), from the same laboratory, reported on the apparent causation of the urogenital agenesis seen by Beaudoin. She noted a failure of the mesonephric duct to connect with the cloaca, as well as degeneration of the metanephrogenic blastema.

In yet another study involving the rat, Kimmel and Fowler (personal communication) found no adverse effects on development following administration of 30 or 90 ppm sodium arsenate or arsenite to the dams in the drinking water throughout pregnancy. The dams were killed and examinations made on gestation day 21.

Additional preliminary work in our laboratory has involved a comparison of the developmental effects of IP versus PO sodium arsenate in mice. Our initial results indicate that single doses of at least 120 mg/kg PO must be used to obtain adverse prenatal effects. Typical results for treatment on day 9 or 10 are shown in Table 4, in comparison with a similar group treated with 40 mg/kg IP. Maternal death rate was similar for both treatments, indicating similar levels of toxicity to the dam. Administration of arsenate IP had a considerably greater effect on prenatal mortality than did PO arsenate, even though the dose was only one third as great. The IP arsenate also decreased fetal weights in comparison with untreated controls, while PO treatment had this effect only when given on day 10. The significant rate of fetal malformation associated with the 40 mg/kg IP treatment is in agreement with our previous observations (*8*). A much lower level of malformation, however, was seen in the orally treated groups.

Table 4. Comparison of the prenatal effects of oral (PO) and intraperitoneal (IP) sodium arsenate in mice.

Treatment[a]			No. of	Dead or	Fetal	Grossly
Day	Dose, mg/kg	Mode	pregnant mice[b]	resorbed fetuses, %	weight, g ± SD	malformed fetuses, %
9	40	IP	10(4)	59	0.80 ± 0.13	34
	120	PO	9(2)	17	0.94 ± 0.07	1
10	40	IP	10(1)	55	0.82 ± 0.13	15
	120	PO	16(2)	26	0.86 ± 0.14	3
None			9(0)	12	1.02 ± 0.11	0

[a]Single dose on gestation day indicated.
[b]Numbers in parentheses indicate maternal deaths.

Our results with orally administered sodium arsenate are in apparent conflict with those of Matsumoto (*11*), who treated pregnant ICR mice on days 9, 10, and 11 with 10, 20, or 40 mg/kg sodium arsenate and examined them on day 18. He reported increased prenatal mortality and decreased fetal weights in the high dose group. In the groups given the 10 or 40 mg/kg doses, a low rate of malformations was also seen (6 and 4%, respectively). Since the number of litters involved was not stated, it is difficult to assess the significance of Matsumoto's findings.

Although arsenite is considerably more toxic than is arsenate, it has received much less attention from teratologists. We treated mice *in utero* with IP injections at dose levels of 10 or 12 mg/kg on one of days 7–12 of pregnancy (*12*). Arsenite treatment resulted in relatively high prenatal mortality, as well as some maternal deaths (Table 5). Treatment on days 8, 9, or 10 induced both gross and skeletal malformations partially similar to but less numerous than those caused by comparably toxic levels of arsenate. Fetal wastage caused by exposure to arsenite was increased in comparison with the level previously seen (*5*) due to arsenate.

Table 5. Effects of sodium arsenite on fetal development in mice: Single IP injections on one of days 7–12 of gestation.[a]

Treatment		No. of	Dead or	Fetal	Grossly
Day	Dose, mg/kg	pregnant mice[b]	resorbed fetuses, %	weights, g ± SE	malformed fetuses, %
7	10	6	51[c]	1.02 ± 0.02	0
	12	7	35[c]	0.91 ± 0.02[c]	2
8	10	6(1)	49[c]	0.81 ± 0.03[c]	8
	12	8(1)	92[c]	0.78 ± 0.04[c]	1
9	10	9	20[c]	0.96 ± 0.01	14[c]
	12	8	78[c]	0.92 ± 0.04[c]	27[c]
10	10	9(1)	88[c]	0.95 ± 0.02	8
	12	6(1)	85[c]	0.57 ± 0.03[c]	36[c]
11	10	9	59[c]	0.95 ± 0.01	0
	12	6(4)	100[c]	—	—
12	10	6(1)	75[c]	1.05 ± 0.03	0
	12	6(4)	100[c]	—	—
Control[d]		36	2	1.05 ± 0.01	0

[a]Adapted from Hood (*12*).
[b]Numbers in parentheses indicate maternal deaths.
[c]Significantly different from the controls ($p < 0.05$).
[d]Controls injected with distilled H_2O on one of gestations days 7-12.

According to the previously discussed results and unpublished data from our laboratory, it appears likely that acute high dose exposure to arsenate is relatively more hazardous to developing mammals than is chronic exposure to only slightly lower daily

doses. If this proves to be the case, the most probable cause lies in the pharmacokinetics involved. Although the pharmacokinetic aspect of the prenatal effects of arsenic exposure is yet to be investigated, it promises to provide interesting answers to the questions posed by differences in effects associated with different routes and modes of exposure. Another aspect of the problem involves the basic cause for the apparent differences in effect between arsenate and arsenite with regard to malformation versus prenatal mortality. This difference would presumably be due to differing mechanisms of action (*13*), but pharmacokinetics may play a role here also. Possible postnatal effects of prenatal arsenic exposure are completely unknown. Answers to the problems thus posed would provide an additional basis for assessment of the potential influence of arsenic on human development.

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